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Transgene-directed immunologic investigations into immune-mediated myositis following delandistrogene moxeparvovec gene therapy

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What does this study mean for the DMD community?

- Peptides derived from micro-dystrophin exons 8 and/or 9 may induce a T-cell response leading to IMM in patients with deletion of these exons. However, not all patients with deletions in exons 8 and 9 develop IMM following gene therapy.
- Knowledge of the patient's HLA presentation of micro-dystrophin peptides, in addition to the patient's specific type and location of pathogenic variant in the *DMD* gene, may help with understanding the immune response to delandistrogene moxeparvovec micro-dystrophin.

Conclusions

- The results indicated that exons 8 and/or 9 of the *DMD* gene appear to be potentially immunogenic; however, not all patients with deletions involving exons 8 and/or 9 develop IMM following gene therapy.
- These data are consistent with those from clinical trials of other investigational DMD gene therapies, suggesting that patients with deletions in specific regions of the *DMD* gene overlapping those expressed in a given micro-dystrophin may be at an increased risk of an IMM event following gene therapy.
- We hypothesize that a combination of the following factors led to IMM in these patients:
  - Deletion of exon 8 and/or 9.
  - Presence of T cells recognizing micro-dystrophin peptides mapping to exons 8 and/or 9 as non-self.
  - HLA type with strong HLA presentation of peptides mapping to exons 8 or 9.
- Work is currently underway to better understand these risk factors and to find ways to safely administer delandistrogene moxeparvovec to patients with potentially higher-risk *DMD* mutations.

OBJECTIVE

- To investigate antigenic features mediating IMM in two patients with DMD treated with delandistrogene moxeparvovec.

BACKGROUND

- Delandistrogene moxeparvovec is an rAAVrh74 vector-based gene transfer therapy, designed to compensate for the absence of functional dystrophin in DMD by delivering a transgene encoding delandistrogene moxeparvovec micro-dystrophin, an engineered protein that retains key functional domains of the wild-type protein.[1–3]
- As of February 2024, delandistrogene moxeparvovec is approved in the USA, UAE, Qatar, and Kuwait for the treatment of ambulatory pediatric patients aged 4 through 5 years with DMD with a confirmed mutation in the *DMD* gene.[4–7]*
- Two serious adverse event cases of IMM were reported in ENDEAVOR (SRP-9001-103; NCT04626674), an open-label, multi-cohort Phase 1b study assessing delandistrogene moxeparvovec in patients with DMD.[8,9]
  - **Case 1** occurred in a 9-year-old patient in Cohort 2 with a deletion of exons 3–43 of the *DMD* gene, 35 days post-dosing. **Case 2** occurred in a 7-year-old patient in Cohort 5 with a deletion of exons 8–9 of the *DMD* gene, 29 days post-dosing. Both patients experienced muscle weakness and received immunosuppressive treatment, including high-dose corticosteroids and tacrolimus.
- In individuals who have a portion of the *DMD* gene sequence deleted that overlaps with the transgene region, there is a risk of the micro-dystrophin being recognized as foreign and, in turn, eliciting an immune response. One requisite of being detected by the immune system is the presentation of peptide fragments of the transgene by HLA-I or HLA-II.† However, not all patients in ENDEAVOR with deletions involving exons 1–17 and/or 59–71 who were treated with delandistrogene moxeparvovec gene therapy developed IMM.
- Here we present the results of the investigation of these two cases.

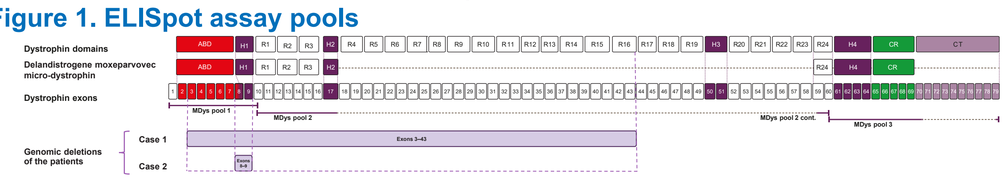
*Delandistrogene moxeparvovec is contraindicated in patients with any deletion in exon 8 and/or exon 9 of the *DMD* gene. †HLA is a complex of genes and proteins that encode and present antigenic peptides to T cells, enabling adaptive immune responses and tissue compatibility.

METHODS

ELISpot assay

- The IFN-γ ELISpot assay was used to detect T cells directed at specific delandistrogene moxeparvovec micro-dystrophin peptides (**Fig. 1**). A combination of peptides from regions of micro-dystrophin was selected to form a peptide pool – MDys pool 1, 2, or 3. The assay detected the specific peptide pools that elicited a T-cell response in the patients. An analysis was performed at the following time points: Case 1 – baseline, Day 2, and Weeks 1, 2, 4, 10, 12, 24, 52, and 104; Case 2 – baseline, Day 2, and Weeks 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 15, 20, and 24.

Figure 1. ELISpot assay pools

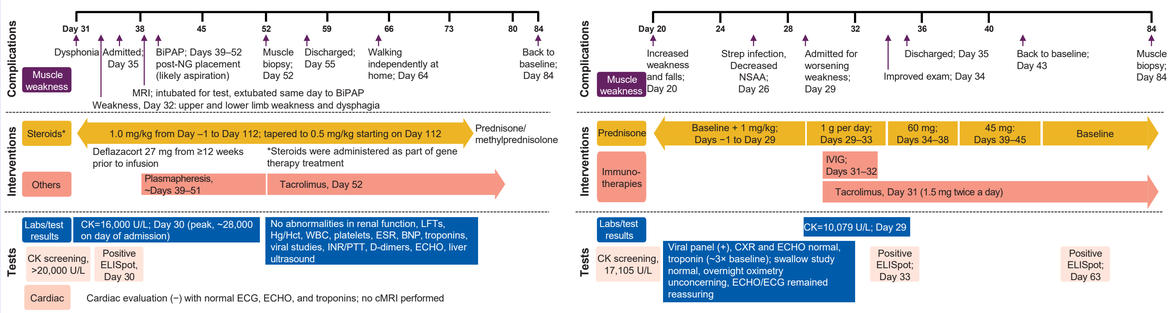


In silico HLA epitope mapping and scoring

- An *in silico* tool (NetMHCpan) was used to determine the propensity of each 9-mer peptide encoded by dystrophin exons 1–17 to bind each HLA-I molecule allele expressed by the patients. Based on the patients' HLA genotypes, individual EL rank values displayed by NetMHCpan were used to calculate "epitope scores" for each dystrophin exon from 1–17. As low EL rank numbers correspond to higher affinities, EL ranks of each 9-mer peptide/HLA allele combination were transformed as follows: [transformed_score = –log2(EL_rank/zygosity^2)] and summed for each exon. The zygosity of the allele, whether homozygous or heterozygous, was accounted for in this transformation.
- Any negative transformed scores were set to 0, establishing an upper EL rank threshold of 1.0 for heterozygous alleles and 4.0 for homozygous alleles.

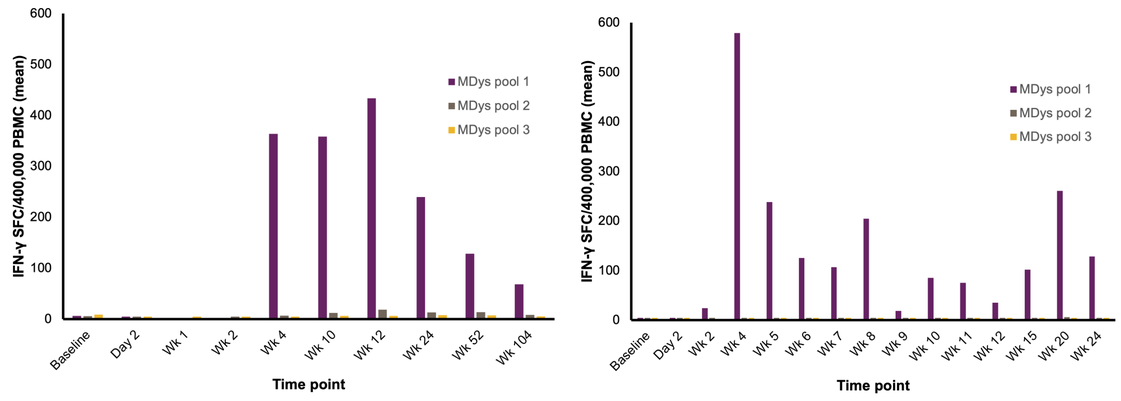
RESULTS

Figure 2. Outcome of the IMM cases



- **Case 1**: The patient underwent six rounds of plasmapheresis and was started on tacrolimus before discharge and completed tacrolimus in January 2024. At discharge (Day 55), the patient did not need any respiratory support, and on Day 67 he regained the ability to walk independently. The patient recovered on Day 100 with sequalae (weakness) (**Fig. 2A**).
- **Case 2**: The patient was started on tacrolimus and IVIG and remains on both. At discharge (Day 35), strength had improved significantly, but per NSAA, was not at his pre-infusion level (**Fig. 2B**).

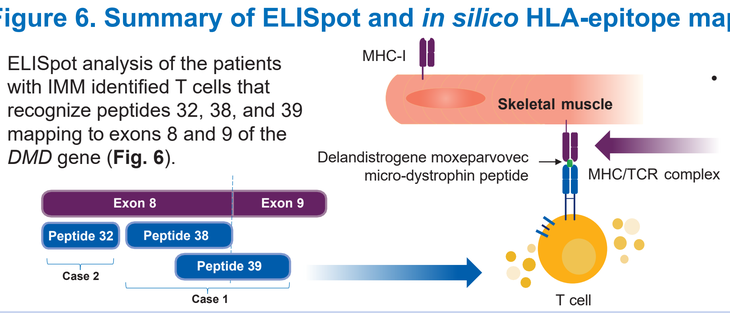
Figure 3. Cellular immune response to micro-dystrophin



- ELISpot analysis suggested that the IMM resulted from T cell-mediated responses directed against specific delandistrogene moxeparvovec micro-dystrophin peptides with elevated responses to peptides from MDys pool 1 (includes peptides from dystrophin peptides 1–10; **Fig. 3**).

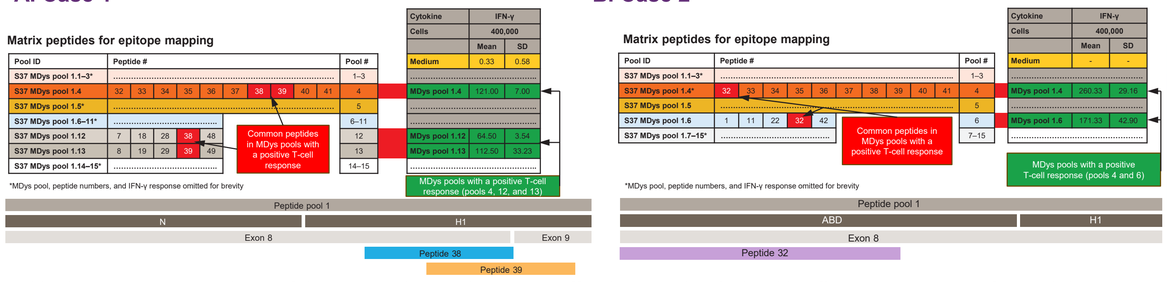
Figure 6. Summary of ELISpot and *in silico* HLA-epitope mapping findings

- ELISpot analysis of the patients with IMM identified T cells that recognize peptides 32, 38, and 39 mapping to exons 8 and 9 of the *DMD* gene (**Fig. 6**).



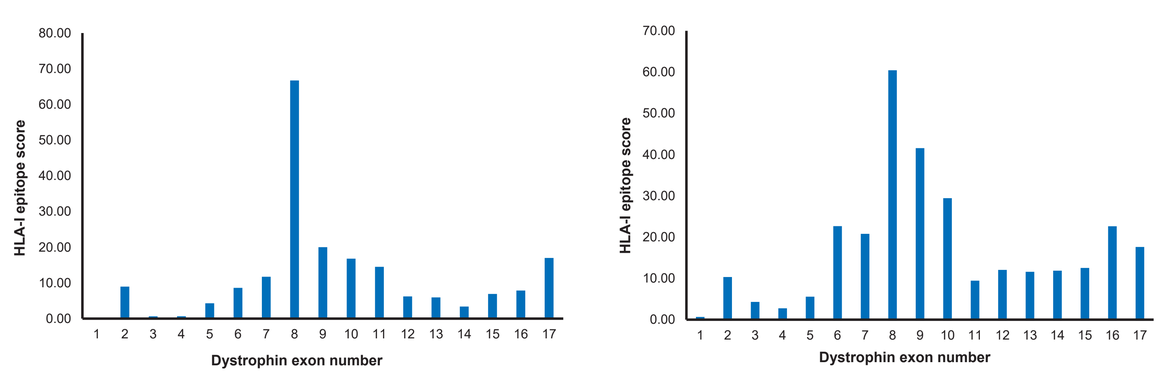
- *In silico* epitope mapping identified peptides encoded by exons 8 and 9 with high propensities to be presented by the patients' HLA-I molecules and the potential to drive an immune response (**Fig. 6**).

Figure 4. ELISpot analysis of micro-dystrophin peptide pool MDys1 to identify potential T-cell targets



- To determine the regions of the micro-dystrophin most effective at stimulating T-cell responses, we cleaved the protein into 51 small peptides, and distributed into 15 pools comprised of varying regions of the micro-dystrophin. We exposed T cells to each pool of peptides and assessed their activation by measuring the amount of IFN-γ produced using ELISpot.
- Upon further analysis, the 51 peptides in MDys pool 1 were grouped into 15 different pools to detect the specific peptides that were eliciting a T-cell response in the patients. ELISpot analysis suggested three peptide pools in Case 1 (in green) of which the common peptides were 38 and 39 (**Fig. 4A**), and two peptide pools in Case 2 of which the common peptide was 32 (**Fig. 4B**), that mounted a T-cell response. Peptides 32, 38, and 39 were identified to induce T-cell responses in ELISpot (IFN-γ secretion) and map to exons 8 and 9 of the *DMD* gene.

Figure 5. *In silico* HLA epitope mapping based on HLA-I scores



- *In silico* analysis of HLA presentation in the patients indicated a greater probability for peptides derived from exons 8 and 9 to bind HLA-I (**Fig. 5**).

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Abbreviations

ABD, actin-binding domain; BiPAP, Bilevel Positive Airway Pressure; BNP, brain natriuretic peptide; CK, creatine kinase; cMRI, cardiac magnetic resonance imaging; cont; continued; CR, cysteine-rich domain; CT, C-terminal domain; CXR, chest X-ray; DMD, Duchenne muscular dystrophy; ECG, electrocardiogram; ECHO, echocardiogram; EL, eluted ligand; ELISpot, enzyme-linked immunosorbent spot; ESR, erythrocyte sedimentation rate; H, hinge domain; Hct, hematocrit; Hg, hemoglobin; HLA, human leukocyte antigen; IFN-γ, interferon-gamma; INR, international normalized ratio; IMM, immune-mediated myositis; IVIG, intravenous immunoglobulin; LFT, liver function test; MDys, micro-dystrophin; MHC, major histocompatibility complex; MRI, magnetic resonance imaging; NG, nasogastric tube; NSAA, North Star Ambulatory Assessment; PBMC, peripheral blood mononuclear cell; PTT, partial thromboplastin time; R, spectrin-like repeat domain; rAAVrh74, recombinant adeno-associated virus rhesus isolate serotype 74; SD, standard deviation; SFC, spot-forming cells; TCR, T-cell receptor; WBC, white blood cell; Wk, week.

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